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COMPARISON OF RADIOFREQUENCY ENERGY AND WARMWATER THAWING OF CRYOPRESERVED RED BLOOD CELLS

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13. ABSTRACT *(Maximum 200 words)*
Units of cryopreserved packed red blood cells (pRBCs) were thawed either with warm water or with radiofrequency (RF) energy at 27.12 MHz and applied with a helical coil system. Cell metabolism, membrane integrity, and hematological parameters were used to assess postthaw cell viability. Results showed that a small amount of RF-induced cell damage due to localized overheating could not be eliminated, but there were no significant differences in the viability comparisons between groups. We concluded that the RF thawing of pRBCs produced no deleterious effects per se, but secondary heat damage prevented this method from being considered a practical modality given the presently available tools.

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SUMMARY PAGE

THE PROBLEM

Currently, frozen red blood cells are thawed in 37 °C water baths. This thawing technique is relatively slow, and a small percentage of the preparations are reported to be pathogenically contaminated during the thawing procedure. The present study compares a dry thawing technique using radiofrequency (RF) energy with the current method.

FINDINGS

The results of this study demonstrate the potential utility of thawing frozen red blood cells with RF energy. The units were subjected to intense RF irradiation, which appeared to produce no direct effect in the individual cells. Deleterious effects were seen only where thermal accumulation could not be controlled.

RECOMMENDATIONS

We believe that changes could be made to reduce or eliminate the overheating problem and improve the RF method. First, the shape of the standard frozen red blood cell bag could be changed to allow even distribution of the RF energy. Second, frozen bags could be mechanically agitated during the RF thawing process to facilitate thermal mixing. Further research using recommended changes should allow a more positive demonstration in the utility of RF thawing of blood units.

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INTRODUCTION

Human whole blood and red blood cell preparations are typically stored for anticipated requirements of patients with blood volume deficiencies or diseases necessitating transfusion. Refrigerated storage of packed red blood cells (pRBCs) at 1-6 °C can maintain cell integrity and viability for up to 35 days, as approved by the Food and Drug Administration (1). Storage of pRBCs at cryogenic temperatures (-65 to -80 °C), on the other hand, can increase clinical availability of whole blood and components up to 3 years for routine use (1). Cryogenic storage has eased the problem of supplying the demand for crucial blood types, even during military operations where the walking donor system has intrinsic problems for the blood bank, recipient, and donor (2).

Conventionally, frozen red blood cells (-65 to -80 °C) are thawed in 37 °C water baths. This thawing technique is relatively slow but otherwise acceptable; although, a small percentage of the preparations may be pathogenically contaminated during thawing procedures (3). The plastic bags used in processing and storage are quite brittle at low temperatures and are subject to damage. This damage allows contamination to occur during warmwater immersion. In a previous study (3), microwave energy (2450 MHz) was used successfully to thaw 3-ml samples of frozen pRBCs as compared to conventional thawing. Our earlier efforts in this area showed that radiofrequency (RF) energy (27.12 MHz), applied with a helical coil applicator, was deposited in a relatively uniform manner throughout most of the volume of a tissue-equivalent full-size model of a pRBC unit (4).

The goal of the present study was to compare RF pRBC thawing with the conventional method using commercially available pRBC units in standard containers. Endpoint comparisons included normal hematological parameters, membrane integrity, and cell metabolism. The results showed no intrinsic RF-induced cell damage although localized thermal accumulation effects were observed.

MATERIALS AND METHODS

APPARATUS

A resonant helical coil was designed to enclose a single frozen pRBC bag for RF dry thawing (4). As shown in Fig. 1, the coil was wound using 11 turns of 0.95-cm OD (outer diameter) copper tubing over a 40-cm length of 25.4-cm OD plastic pipe that had been heated and pressed into an oval shape. The last turn of the coil was attached to the main coil by a 3-cm section of flexible braid to allow tuning to aid in maintaining the coil/sample system at resonance during the thawing process. Radiofrequency energy was coupled to the main coil by a primary winding, consisting of 5.5 turns of 0.63-cm (OD) copper tubing. The primary winding was centered and supported longitudinally over the main coil on 0.48-cm diameter glass rods. The coil system was partially shielded by a sheet of grounded aluminum foil taped to a 1.65-mm PVC plastic form to reduce stray irradiation. Styrofoam blocks (2.5 by 2.5 by 30 cm) were used to centrally support the cardboard storage box containing a frozen pRBC bag. Similarly, styrofoam blocks were used to support the coil assemblage inside the shield.

Radiofrequency energy at 27.12 MHz, continuous wave, was supplied by a military URT23B communications transmitter. A 2-m length of RG-5B 52.5 ohm coaxial cable connected the transmitter to the primary winding. Forward and reflected RF power levels were monitored at the transmitter output with a Bird Model 43 directional power meter.

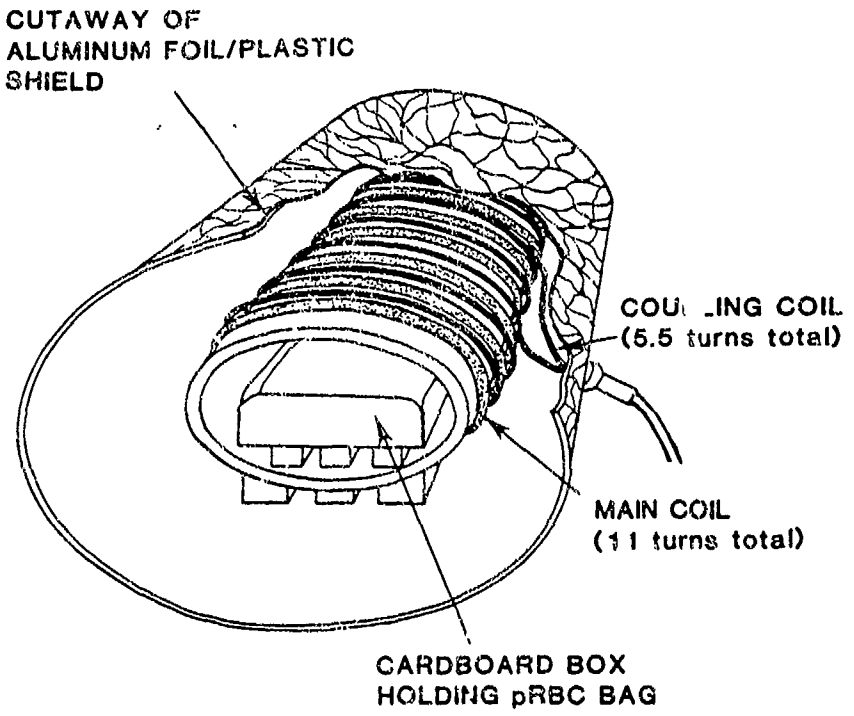

Figure 1. Diagram of the RF coil system.

Twelve standard 800-ml collection bags (Fenwal #4R1242) individually packaged in standard 18- by 13- by 4-cm cardboard storage containers were provided by the Naval Blood Research Laboratory, Boston University School of Medicine, Boston, Massachusetts. The bags, which contained approximately 350-450 ml of glycerolized pRBCs, were received frozen and then immediately stored at -80 °C. Ten bags were used in five paired water-bath and RF thaw experiments; two bags were used for preliminary tuning and testing studies.

PROCEDURE

Preliminary RF heating experiments were conducted to optimize the coil system for use with actual pRBC samples. In a previous study, relatively high RF energy deposition occurred in the folded flap region of a sample bag filled with tissue-equivalent material (4). One aim of the preliminary experimentation, therefore, was to provide an RF power level for the comparison experiments that would be the best compromise between thaw time and localized heat damage. We found that as thawing progressed, a large change in electrical properties of the system occurred, as indicated by increased reflected power. To reduce this reflection and maintain resonance, the circuit was manually tuned by adjusting the position of the last turn of the coil. As the pRBCs continued to thaw, this procedure no longer produced resonance. Subsequently, the bag was positioned closer and closer toward one end of the coil to reduce the reflected power. We attempted to reduce the effects of uneven thawing by periodic rotation and inversion of the blood bag. When RF output power was limited to 125 W and was interrupted for about 30 s at 5-min intervals, pRBC damage was limited to a relatively small volume in the flap region and the bag seam. We selected this power level and procedure even though the RF thaw time has increased to approximately 75 min.

RF THAW

Thawing experiments were conducted as five paired experiments on different days. The pRBC bag selected for RF thaw was removed from the

freezer and placed inside the coil. The transmitter was energized and set for an RF power level of 125 W followed by adjustment of the main coil until a wattmeter indicated reflected power of 5 W or less. At that time, RF energy was applied as described in the preliminary experiments. As the bag became pliable, toward the end of the thawing period, it was manually palpated to promote thermal mixing of the cells. Radiofrequency heating was discontinued when the surface temperature of the bag reached 5.0 °C as measured with a Hewlett Packard Model 2802A digital thermometer. The average thaw period for the five RF samples was 75 min.

WATER BATH THAW

A second pRBC bag was removed from the freezer approximately 45 min after its RF comparison bag to permit parallel, simultaneous processing of thawed samples and thereby allow for the difference in thawing time between methods. To start each experiment, the frozen pRBC bag was removed from the cardboard storage container and then placed in a 37 °C circulating water bath. As the bag became pliable, it was palpated in the same manner as the RF thaw procedure. Surface temperature of the bag was measured with a Sensortek Model BAT-12 digital thermometer. Thawing was discontinued when the surface temperature of the bag was between 5 and 12 °C. The average water bath thawing time for the five experiments was 21 min.

POSTTHAW

A 12-ml aliquot was taken from the thawed pRBC bags and placed in a sterile 40-ml screw-capped polypropylene vial for each experiment. All aliquots were taken from a bag region containing undamaged cells. Each of the samples was temperature equilibrated to 20 °C in a water bath before starting the washing procedure. The samples were washed using similar commercially prepared solutions and procedures developed by Valeri (5) and modified by Campbell (3). Samples were then incubated in a 37 °C water bath for up to 4 h depending on which test followed.

METABOLISM

Glucose consumption and lactate production over a 4-h interval were used to assess cell viability. One aliquot of the incubated pRBCs was removed from the water bath, placed in a test tube, and analyzed to start the procedure. This step was repeated at the end of each successive 1-h period until 4 h of cell metabolism had been completed. Assayed glucose concentrations were determined by the hexokinase method (Sigma Diagnostics, procedure no. 115), and utilization levels were measured spectrophotometrically (Beckman DB 25) to monitor RBC metabolism. The lactate determination procedure also utilized an enzymatic technique (Sigma Diagnostics, procedure no. 726-uv/826-uv).

MEMBRANE INTEGRITY

Qualitative measurement of sodium uptake by the thawed pRBCs was used as an indication of the membrane integrity. We used a method similar to that described by Liburdy and Penn (6). Deglycerolized erythrocytes from both conditions were resuspended to approximately 25% hematocrit and apportioned to two 1-ml aliquots for each condition. The resulting four samples were divided into active and passive transport preparations for sodium uptake determination. All four aliquots were labeled with 0.2 ml of 1.25 microcuries ^{22}Na/ml radioisotope and incubated for 1 h in a 37 °C water bath. A gamma counter (Packard Instrument 5500) registered the total counts-per-minute activity in order to correct the results to a common activity for each aliquot. Samples were then centrifuged at 4000 g for 2 min. The supernatant was removed, and cells were resuspended in a 1-ml wash solution of lactated ringers with 11 mM glucose and 0.01% bovine serum albumin. After a second centrifugation, the supernatant was decanted, and 0.2 ml of packed pRBCs were

transferred to a tube for a cell activity count. Aliquots designated for the comparison of passive sodium transport received an initial 90-min incubation with 0.005 ml ouabain (0.1 mM) at 37 °C in addition to these procedures.

HEMATOLOGY

Hematological analyses included hematocrit (HCT) (Hct Reader), hemoglobin (HGB) (Coulter Hemoglobinometer), red blood cell (RBC) count, and mean corpuscular volume (MCV) (Coulter ZM Particle Counter). Because these analyses used samples that were also initially resuspended to approximately 25% hematocrit, both HCT and RBC were not objectively meaningful.

DATA ANALYSIS

A two-tailed t test was used to compare the hematological and membrane integrity results. A split-plot analysis of variance (ANOVA) was used to compare the serial metabolism results. In all comparisons, the significance level was at $p = 0.05$.

RESULTS AND DISCUSSION

Visible evidence of cell damage from localized heating was found in all of the RF thawed pRBC bags, but care was taken to ensure that the aliguot for analysis was obtained from an undamaged region. Hematological results are given in Table 1.

TABLE 1. Means (±SD) of Hematological Parameters.

Method of Thaw	HCT (%)	RBC ($10^6/\mu l$)	MCV (μ^3)	HGB (g/dl)
Water	26.2 ±1.8	3.5 ±0.3	103.2 ±8.0	9.8 ±0.8
RF	25.6 ±3.1	3.5 ±0.6	94.8 ±10.6	9.5 ±1.5

Glucose utilization of the RF group corresponded closely to that of the warm water group (Fig. 2). Lactate production was also similar as shown in Fig. 3. A split-plot ANOVA comparison showed no significant difference. The membrane integrity assays of both passive and active sodium transport indicated only a slight variation in the means (Fig. 4), and no statistical difference was observed.

These results demonstrate the potential utility of RF energy in thawing units of pRBCs. We readily acknowledge the limited extent to which we can generalize this utility because of the localized overheating problems that could not be entirely eliminated. Although we found no evidence of intrinsic RF-induced cell damage, the helical coil applicator system, as presently configured, cannot be considered to be a practical device. The pRBC units were, however, subjected to intense RF irradiation, which appeared to produce no direct effect in the individual cells. Only where the thermal accumulation could not be controlled were deleterious effects seen.

We believe that several changes could be made to reduce and/or eliminate the overheating problem. First, the bag could be reshaped to eliminate the folded flap region and thereby eliminate the regions of high energy absorption that were seen in our previous study (4). Second, the pRBC unit could be agitated mechanically during the RF thawing process to facilitate thermal mixing. Third, in the frozen state, pRBCs are characterized as having a very low dielectric loss factor as compared to the liquid state, therefore, the

large change in electrical properties of the pRBCs from the solid to liquid phases could be better accommodated by using either two irradiation frequencies or two types of applicators for a given frequency.

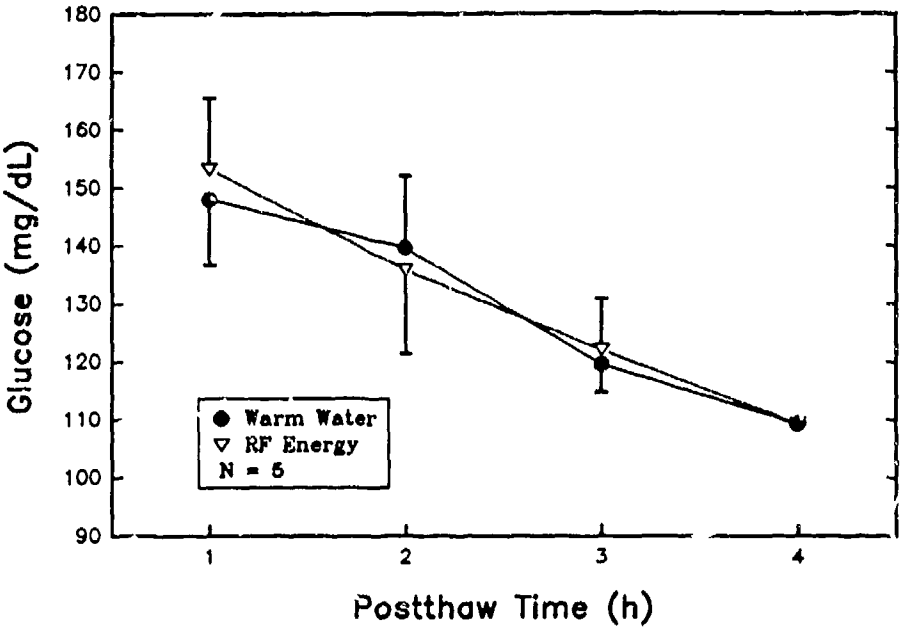

Figure 2. Mean = ± SD glucose concentration after thaw.

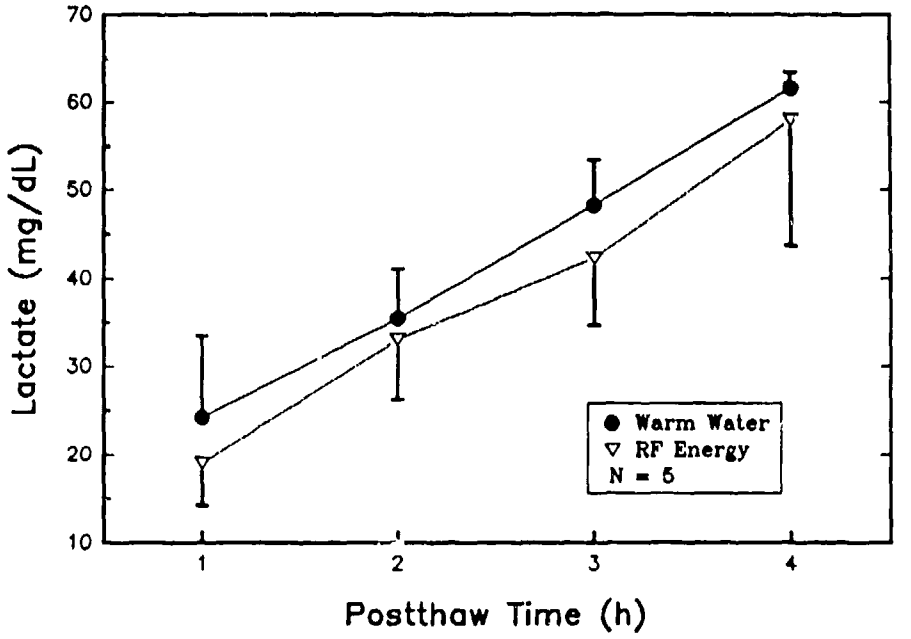

Figure 3. Mean = ± SD lactate concentration after thaw.

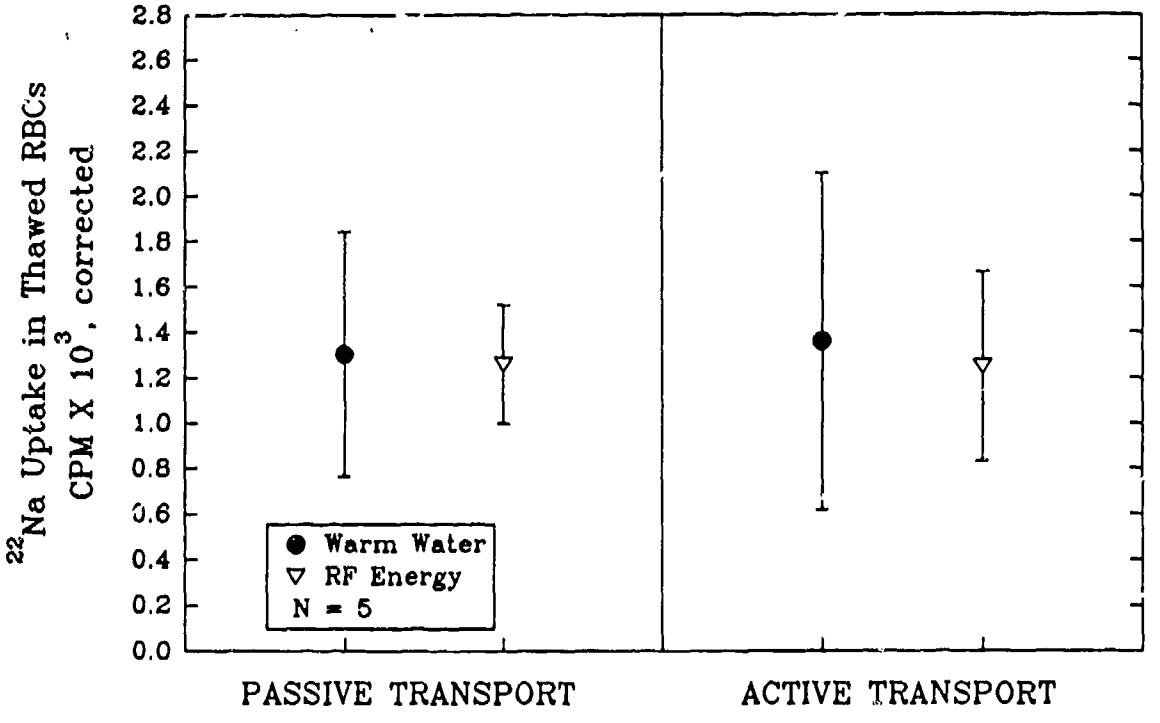

Figure 4. Comparison of membrane integrity results (mean ± SD).

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